DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Memorandum

Food and Drug Administration
Center for Biologics Evaluation and Research
Bethesda, MD 20892

Date : 5/6/97

To : File

From : Mark Brunswick, Division of Monoclonal Antibodies - HFM 555

Through : Division Director, Division of Monoclonal Antibodies

Subject : BLA 97-0244

THERAPEUTIC AGENT(S): Rituximab (C2B8)
Chimeric human/mouse antibody, human heavy chain constant region (Fc domain) and murine heavy and light chain variable regions (Fab domain)
IgG1 κ
The target antigen is human CD-20 on human B cells

SPONSOR(S): Genentech, Inc
460 Point San Bruno Boulevard
South San Francisco
California 94080-4990

CLINICAL INDICATION(S): Treatment of patients with relapsed or refractory low-grade or follicular B-cell non-Hodgkin's lymphoma (indolent lymphoma)

This application was submitted in conjunction with IDEC BLA 97-0260, and is for fill and finish of Rituximab (IDEC-C2B8) anti-CD20 monoclonal antibody. This application contains all of the information for final lot release of Rituximab and of stability studies done on the final product including shipping stability. The application also contains detailed information on the facility and an environmental assessment these will be reviewed in detail by the DEL review team.

The application consists of 4 volumes, volume 1 is a summary volume that is the same as the summary volume submitted by IDEC, as such it will not be reviewed in this memo but with the IDEC submission. The other 3 volumes contain sections on: drug substance, drug product, investigational product formulation (the information for this section is found in IDEC's BLA 97-0260), environmental assessment and method's validation (drug product) information.

The Genentech parenteral manufacturing facility is utilized for the filling/ lyophilization of all of Genentech's commercial drug and biological injectable products. The facility is licensed under Genentech license number 1048. The facility is operated as a multi use facility and an application was filed in September 1995 as an ELA supplement to allow the introduction of clinical products into the facility. The ELA supplement included cleaning validation studies and procedures, practices and controls that address the prevention of product cross-contamination, and describes the control procedures for the introduction of new products into the marketed product manufacturing facility. The ELA supplement 95-1521 contained SOP [~~~] for the introduction of new products into the facility, the supplement was approved in November 1995. The SOP [~~~] for the introduction of new products into the facility is also included in the current BLA Application.

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Rituximab is received from IDEC as a formulated bulk that has been [~~~] filtered into pre-sterilized [~~~] bags and shipped [~~~] [~~~] from IDEC to Genentech. The purified IDEC-C2B8 is prepared as a liquid formulation [~~~] The pH of the formulation is adjusted to 6.5 [~~~] The process flow is described in the attached process flow chart, attachment 1, and will be described briefly in this review. The total hold times have been validated and are described in Table B.7.b.2-1, attachment 5.

The product is filtered into a steam-sterilized stainless steel [~~~] freeze/thaw tank. The product is sampled for endotoxin and bioburden prior to filtration and protein concentration after filtration. The filtered bulk may be frozen if required, hold times and number of freeze/thaw cycles has been validated and is shown in the attached table B.8.a-3, attachment 8. The [~~~] hold tanks are pooled to give the desired batch size, which is either pooling into [~~~] tanks, the pooled bulk may be held for 48 hours at 2-8°C. This time has been validated and the two days at 2-8°C is included in the 60 day maximum hold period at 2-8°C and has been validated for both fresh and frozen product, table B.8.a-3, attachment 8.

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The product is filled as either 10cc or 50cc sterile, depyrogenated USP Type I borosilicate glass vials with 20mm gray butyl rubber liquid stoppers and sealed with a 20mm aluminum/plastic flip-off type cap. The vial specs, including all vial dimensions, are included in the application and are purchased either from [~~~] Glass or [~~~] stoppers are gray butyl rubber from [~~~] The process gas is Nitrogen. Vials are then stored for inspection and release by quality assurance and quality control. Filled vials are 100% manually inspected prior to labeling. Vials are then labeled and released for shipping after release by quality assurance. The storage time in the vials is 24 months from the time filling, this time includes the storage prior to release by quality assurance.

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Drug Product Stability:

The sponsor has done extensive stability studies on the product, the studies are well controlled and the results are summarized below.

The clinical lots met all specifications after storage for 24 months at 2°C-8°C.

No difference was observed for products stored in 10cc vials or 50cc vials.

The orientation of the vial (inverted or upright) had no effect on the stability of the product.

An analysis of product made from either master cell bank in the presence or absence of transferrin showed the products were comparable. In that no differences were obsereved in any of the stability parameters examined. Parameters that were examined were: pH, color and appearance, UV spec, SDS-PAGE, SEC, fragment IEC, huCDC.

No effect on drug stability was observed if the product was diluted either in 0.9% saline or 5% dextrose. In addition the composition of the intravenous bag, polyvinylchloride or polyolefin, had no effect on the product. In a study to examine the effect of exposure of the product to 24 hours of intense light under a sunny widow showed no effect on the product.

Long-term (over 3 days) exposure of the product to intense light should be avoided. Storage for longer periods causes a breakdown of the product as is evidenced by increased fragmentation and a decrease in a rabbit complement dependent cytotoxicity assay.

Worse case scenarios for shipping conditions including ambient temperature and continuous agitation showed the product would survive shipping.

The proposed 24 month expiration date is supported by the data.

Lot release tests

Lot release testing is done by both IDEC on the formulated bulk and Genentech on the final product, below are the tests performed by both sponsors.

IDEC	Genentech
Color and Appearance	Color and Appearance
Peptide Map	Particulates
SDS-PAGE	Container Volume
Fragment IEC-HPLC	Cappilary IEF
SEC-HPLC	SEC-HPLC
Glycan Content	
LAL	Endotoxin
Bioburden	Sterility
Potency-CDC	CDC
Strength-UV	UV spec scan
pH	pH
Polysorbate 80	Osmolarity

Complement dependent cytotoxicity:

(b)(4) One of the lot release assays is a human complement dependent cytotoxicity, this complement is currently purchased from [redacted] An SOP [redacted] is included [redacted] for the qualification of additional lots as well as the preparation of a source of complement from volunteer donors. The lots are

qualified using C2B8 dependent cytotoxicity.

The sponsor has validated the complement dependent cytotoxicity assay in a very detailed manner. Some of the parameters that were found to be critical were: the lot and dilution of human complement, and the galactose content on the heavy chain (and as such, one of the lot release specs from IDEC is to assay for glycan). Items that were found to have very little effect on the assay include: cell passage number (the cell line used for the assay is the human B-lymphoblastoid cells, [~~~] cell density, cell suspension storage time, assay incubation time, FCS, BSA, tissue culture plates and the source of human complement.

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The sponsor in conjuction with IDEC have shown that while the binding of Rituximab to CD-20 is unaffected by the galactose content the complement dependent cytotoxicity assay is affected by the galactose content. These galactose molecules are on the heavy chain of the chimeric molecule. Below is a graph taken from the IDEC submission which shows how the number of galsctose molecules (0-2 moles/mole of heavy chain) will affect the complement dependent cytotoxicity assay giving a result of 80-150% of the maximum depending on the galactose content.

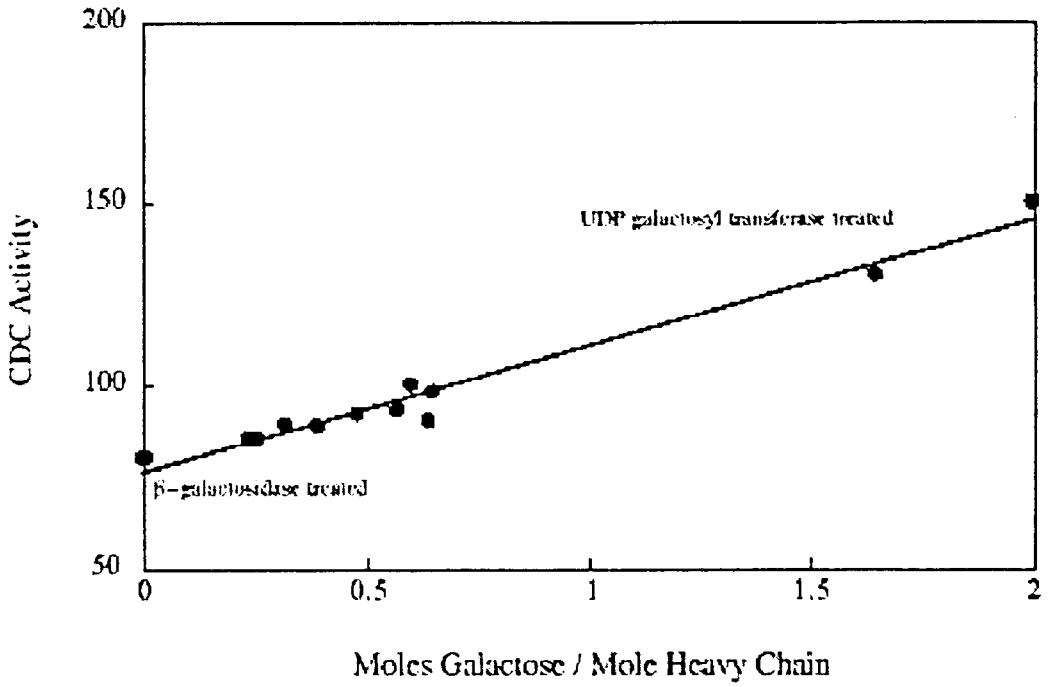

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Figure A.1.b.2.-9. Correlation of CDC activity and galactose content for IDEC-C2B8.

[

6 lines

] The data were fit using a linear least squares fitting

program. Moles galactose are calculated as: G0 = 0 moles; G1 = 1 mole; and G2 = 2 moles galactose.

(Figure copied from IDEC BLA 97-0260)

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The sponsor has examined a large number of parameters associated with the cell line [~] The sponsor has a master cell bank for this cell line although no information is submitted. They have studied the number of passages that the cell vial is useful [

4 lines

] Another parameter that was examined was the cell density used for culturing the cells, cells could be cultured cell that at the end of culture they are at a concentration of [~] cells /mL. The cells are maintained in culture until they are used for the assay.

The assay demonstrated an overall accuracy of 97-102% accuracy, with an overall relative standard deviation of 7%. The largest contribution to this error was found to be well-to-well variability. The assay was found to be accurate with a working range [~]

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Five different lots of Rituximab have been tested in this assay and all have had similar results. The reference material is lot C2B81095-1, the potency was arbitrarily assigned a specific activity [~]

One of the controls that Genentech presents is the storage conditions to be used on the complement once it is thawed. The sponsor presents evidence that the complement is stable for up to 5 hours whether stored at room temperature or at 2-8^{0}C. The recommended procedure is to use freshly thawed complement, although in light of this result they do allow for complement use for 5 hours after it has been thawed provided that it is stored at 2-8^{0}C.

Ultraviolet-visible spectrophotometric scan:

Since one of the lot release tests is protein concentration, based on ultraviolet spectrophotometric scan, the sponsor needed to validate the assay and to get an accurate extinction coefficient in order to accurately estimated protein concentration. The sponsor used protein analysis by amino acid analysis and protein concentration by [~] to compare to the ultraviolet scan. The results showed that the extinction coefficient of Rituximab is 1.7 ml/(mg x cm).

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Environmental Assessment:

The filling of this product at Genentech's South San Francisco facility should have no adverse effect on the environment. The sponsor has given a detailed account of what will be released into the environment and the controls that they will be using to reduce the environmental impact.

Some production materials that will be released to the publicly owned treatment works are adjusted for pH prior to release. Hazardous wastes will be shipped off-site.

Conclusion:

Genentech is able to fill Rituximab in a consistent manner yielding a potent product that meets the specifications described in the BLA application.

Comments to the Sponsor:

1) Biological products are required to have a test for rabbit pyrogenic substances as described in 21 CFR 610.13(b) on the final product. This test may be replaced by the Limulus amoebocyte lysate assay after this assay is shown to be equivalent for this product (21 CFR 610.9). In the application, the validation of the LAL test is given but not the comparison to the rabbit pyrogen test. Please submit these data for at least one lot. After the data are submitted and reviewed the LAL test may be used as a routine lot release assay.

2) The silver stained SDS-PAGE gels that were used in the stability studies were not included in the Application. Please submit the gels to the file.

(b)(4) 3) Please submit the test procedure for determining fragment IEC, SOP [redacted]

4) The process gas is nitrogen but is this also the gas in the head space of the vials nitrogen or is it air? Is the nitrogen filtered and sterile?

5) The product is stored for up to 60 days in polyolefin bags, which are also the bags used for the infusion. Have any assays been performed on the product to assess the presence of leachables from the polyolefin bags?

6) The product is shipped from IDEC in [redacted] bags and is then transferred to [redacted] freeze/thaw tanks. What volume of product is transferred to these tanks?

7) Please submit the information on the generation of the master cell bank for [redacted] cell line and a fuller discription of the cell line.

Attachments:

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(These are copied from the application and have the volume and page number in the upper right corner)

1) Process flowchart showing C of A testing/in process controls, process flow, hold times and transfer method.

2) Statement of composition, given in per ml and per 11 for the batch.

3) Specifications and methods: the tests, SOP (test) code, SOP method and specifications. In volume [redacted] all the SOP's are submitted in the Application and have been reviewed.

4) Release Specifications, and summary for results for lots D9097A, D9098A

and D9099A.

5) Bulk holding times: Holding step, vessel, temperature and time.

6) Batches tested for stability.

7) Stability protocol.

8) Freeze/thaw bulk stability results on Lot 0128.

9) Comparability studies on 4 lots from 2 different Master Cell Banks ± transferrin.

10) Final vial testing including different vial sizes, different filling sites, different master cell banks with or without transferrin.

11) The stability protocol for commercial Rituximab drug product lots.

12) Stability of lot 0111-5, 10cc vial configuration, date of manufacture 1/24/95.

13) Stability of lot 0112-5, 10cc vial configuration, date of manufacture 2/9/95.

14) Stability of lot 0112-6, 50cc vial configuration, date of manufacture 1/31/95.

15) Stability of diluted Rituximab in either 0.9% saline or 5% dextrose. The table has information on the i.v. bag composition, i.v. solution, and light exposure.

16) Rituximab stability in the infusion apparatus. The table has information on infusion rate, target concentration, presence of an in-line filter, and when during the infusion the sample was taken.

17) Photostability of Rituximab drug product. Shown are the number of days, total light exposure. Assays used are; fragment IEC, size exclusion chromatography and a rabbit complement dependent cytotoxicity.

18) Shipping study for Rituximab. The table shows the temperatures used for shipping the product and then storing it as well as whether there was any agitation during the storage. Testing was; pH, color and appearance, UV spec, SDS-PAGE (silver stain), size exclusion chromatography, fragment isoelectric focusing, complement dependent cytotoxicity, particles and container closure.

19) The ability of capillary isoelectric focusing to positively identify IDEC-C2B8 from other monoclonal antibodies made by IDEC.

20) The ability of capillary isoelectric focusing to positively identify IDEC-C2B8 from other monoclonal antibodies made by Genentech.

21) The effect of storage time and temperature on complement activity.

22) Specificity of the complement dependent cytotoxicity test. The table shows that none of the products marketed by Genentech have any activity in this assay except Rituximab.

23) Effect of Genentech products on quantitation of Rituximab in the complement dependent cytotoxicity assay. None of the products had any effect on the quantitation of Rituximab.

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THIS SECTION WAS DETERMINED TO BE NOT RELEASABLE

24 pages